Presurgical Imaging with Indium-labeled Anti-Carcinoembryonic Antigen for Colon Cancer Staging

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Abstract

Over a 4-year period, 108 patients with known or suspected colorectal cancer were studied by radioimmunoconjugate scintigraphy prior to operative procedures. Study subjects received 0.2 to 40 mg i.v. of murine anti-carcinoembryonic antigen monoclonal antibody labeled with 2-5 mCi of 111In (Indacea). Resected tissues were analyzed for 111In and carcinoembryonic antigen content. Tumor, liver, and draining lymph nodes had over 10% injected dose/kg compared to <2.5% injected dose/kg for other normal tissues. Primary tumors that were successfully imaged were significantly larger and had higher 111In and carcinoembryonic antigen content. In 54 patients, primary tumors were visualized with a sensitivity of 78%. Hepatic metastases (58 patients) were visualized as negative filling defects (sensitivity, 45%). Extrabdominal (intraabdominal) metastases (25 patients) were visualized (sensitivity, 48%) as areas of increased uptake. Extrabdominal metastases were uncommon (10 patients; sensitivity, 80%). Of 56 patients with known or suspected hepatic metastases who presented with no evidence of extrahepatic disease by conventional tests (X-ray, computerized tomographic scan), 20 (36%) were documented to have extrabdominal metastases at exploratory surgery and 10 of these (50%) had the extrabdominal disease localized by the Indacea scan. The management of these 10 patients was, or could have been, modified by the scan findings and unnecessary surgery eliminated.

Introduction

CEA is a well characterized tumor-associated antigen found in over 95% of colorectal adenocarcinomas (1). Antibodies to CEA have been produced and radiolabeled for targeting to tumor tissue. Early studies used polyclonal antibodies and 111In labeling (2-6), but more recently murine MABs and 111In have been utilized (7-12). We previously reported our experience using a high affinity 111In-labeled MAB (Indacea) directed against CEA (12, 13). Observations in 40 patients were validated by surgical exploration.

We have now expanded the study to 108 patients with colorectal cancer who have been imaged and gone on to a surgical procedure for diagnosis and/or treatment. In this report, the association of tumor imaging with various tissue parameters is documented. The effectiveness of primary tumor and metastatic tumor imaging has been analyzed and a role for Indacea scintiscanning in the management of colorectal cancer patients has been identified.

Materials and Methods

Indacea. As previously described by Paxton et al. (14), the N-hydroxy succinimide active ester of DTPA was synthesized and conjugated to T84.66, an IgG1 monoclonal antibody specific for CEA. 111In was incubated with the antibody conjugate at a ratio of 10 mCi/µg to form Indacea. A second anti-CEA IgG1 MAB was produced by Hybritech (San Diego, CA) and designated ZCE025. This MAB was purified from murine ascitic fluid by an 18% sodium sulfate fractionation, followed by DEAE-Sephalic ion exchange chromatography. The ZCE025 was conjugated and labeled with a proprietary DTPA bifunctional chelate technique at a ratio of 5 mCi/µg. In the case of each MAB, excess EDTA or DTPA was added to the reaction mixture (Indacea) prior to patient administration to complex free 111In and facilitate renal excretion of the unbound radioisotope. All MAB utilized was sterile, non-pyrogenic, and without evidence of murine viral contamination. Each antibody was prepared and administered under Food and Drug Administration approval (T84.66, BB-IND-2014; ZCE025, BB-IND-2041).

Patients. Patients eligible for the study were those: (a) with previously untreated colorectal carcinoma in whom laparotomy was planned for bowel resection; (b) with hepatic metastases of colorectal carcinoma in whom laparotomy was planned for hepatectomy and/or for continuous infusion pump placement; and (c) previously having had "curative" resection for colorectal carcinoma in whom an elevated serum CEA developed and in whom a "second look" laparotomy was planned. Patients signed informed consent prior to participation in the study. The study and consent procedures were approved by the Institutional Review Board for the City of Hope National Medical Center.

Scintiscanning. Radiolabeled T84.66 (200 µg, 2 mCi) was administered by direct i.v. infusion. 111In-ZCE025 (1.0 mg, 5 mCi) was mixed with 19 or 39 mg of unmodified MAB (ZCE025), and infused i.v. over 30 min in 100 ml of saline. Multiple anterior and posterior scintiscan images of the trunk were obtained at least twice (24, 48, 72, 120, or 168 h) using a Technicare Omega 500 camera and a medium-energy collimator. Scintillation data were stored in a Technicare 560 computer.

Surgery. Patients underwent surgery 5 to 20 days after the MAB injection. If suspicious areas were noted on scintiscans, additional noninvasive diagnostic testing and/or transcutaneous biopsy was attempted to confirm the presence or absence of disease, at the discretion of the patient’s physician. In cases in which the presence of extrabdominal metastasis was suggested, additional appropriate operative procedures were similarly utilized to confirm suspected disease.

When surgical procedures were performed, a careful examination of all accessible regions identified any previously unsuspected disease. In those patients with suspicious uptake on CEA scan particularly careful examination and appropriate tissue sampling were utilized to confirm or reject the diagnosis of extrahepatic disease. Tissues removed in the region of "hot spots" were subjected to careful gross and microscopic pathological analysis as well as evaluation of radioactivity content and CEA content.

Tissue Analysis. Tumor and other tissue removed at operation were examined in standard fashion by the surgical pathologist as described previously (12). Aliquots in excess of that required for histopathological analysis were weighed and analyzed quantitatively by enzyme immunoassay for CEA content and by gamma counter for 111In content. The Roche enzyme immunoassay kit (a gift from Hoffman La Roche Inc., Nutley, NJ), used for measurement of CEA content, employs the T84.66 antibody. For each tissue specimen the 111In content was corrected for radiation decay and expressed as percentage of injected dose/kg of tissue. Mean values ± SE, median values, and range have been calculated. The observed data for the tissue analysis parameters were skewed toward large values and thus statistical comparisons were performed using the Wilcoxon rank-sum test.

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2The abbreviations used are: CEA, carcinoembryonic antigen; MAB, monoclonal antibody; Indacea, 111In-labeled anti-CEA MAB; TP, true positive; FP, false positive; TN, true negative; FN, false negative; CT, computer-assisted tomography; DTPA, diethylentriaminepentaacetic acid.

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Results

Between June 1984 and June 1988, 108 evaluable scintiscan studies were undertaken in colorectal cancer patients and subsequently followed by an operative procedure for diagnosis and/or treatment. Four patients had two Indacea studies, each study prior to a different operative procedure. Sixty-five scintiscans followed administration of 200 µg of $^{111}$In-labeled T84.66 and 43 followed 20 to 40 mg of $^{111}$In-labeled ZCE025. The 200 µg of T84.66 were labeled with 2 mCi of $^{111}$In and scans were performed at 24 and 48 h. The 20 and 40 mg of ZCE025 were labeled with 5 mCi of $^{111}$In. These Indacea scans could be performed at 48 to 72 h and 5 to 7 days because of the higher dose of radiolabel.

The individuals responsible for scintiscan interpretation (J. D. B., D. Y.) were provided with relevant clinical data such as location of known primary or metastatic tumor at the time of scan evaluation. The operating surgeon was notified immediately regarding areas of increased Indacea uptake that did not correspond to known tumor locations. The operative procedures were undertaken so that suspected tumor sites were examined and biopsied, provided this was in the best interest of patient care. Postoperatively, the extent of disease and scan status were compared in each of four categories: primary tumor; hepatic metastasis; extrahepatic abdominal metastasis; and extraabdominal metastasis. In each category, the scan was scored as either positive or negative and, on the basis of the surgical/pathological assessment, each scan study was recorded as one of: TP, FP, TN, or FN. In each category, the Indacea scintiscan sensitivity [(TP/(TP+FP)], specificity [(TN/(TN+FP)], and predictive value [positive scan, TP/(TP+FP); negative scan, TN/(TN+FN)] were calculated.

Scintiscan Accuracy. We analyzed scintigraphy findings for primary tumors by individual tumors so that tissue analysis parameters could be compared for imaged (TP) and nonimaged (FN) lesions. We analyzed metastases for their presence or absence by region (hepatic, extrahepatic abdominal, extraabdominal) as treatment decisions were generally based upon presence or absence of disease in these areas, not the exact lesion number in an area.

Thirty-six (78%) of 55 primary adenocarcinomas of colon or rectum in 54 patients were visualized as positive images by Indacea scintigraphy (Table 1). Positive Indacea scintiscans had an excellent predictive value (100%) for the presence of primary tumors (Fig. 1) but were not able to distinguish between luminal tumor (primary) and extrahepatic abdominal metastasis.

Fifty-eight patients had one or more hepatic metastases from colorectal adenocarcinoma of which only 26 (45%) were visualized by Indacea scintigraphy. The extensive uptake by histologically normal liver (Fig. 1) resulted in large or extensive metastases appearing as photopenic areas (Fig. 2). On several occasions, a hepatic metastasis had increased uptake relative to the normal liver. Differentiation of hepatic metastasis from extrahepatic metastasis or histologically normal retroperitoneal lymph nodes adjacent to the liver was often not possible using planar Indacea scintigraphy (Fig. 3).

Table 1 Accuracy of INDACEA scintigraphy of colorectal cancer operative correlation

<table>
<thead>
<tr>
<th>Tumor location</th>
<th>True positive</th>
<th>False positive</th>
<th>True negative</th>
<th>False negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumor</td>
<td>43</td>
<td>0</td>
<td>54</td>
<td>12</td>
</tr>
<tr>
<td>Hepatic metastasis</td>
<td>26</td>
<td>1</td>
<td>49</td>
<td>32</td>
</tr>
<tr>
<td>Extrahepatic metastasis</td>
<td>12</td>
<td>7</td>
<td>76</td>
<td>13</td>
</tr>
<tr>
<td>Extraabdominal metastasis</td>
<td>8</td>
<td>2</td>
<td>96</td>
<td>2</td>
</tr>
</tbody>
</table>

Twenty-five patients had metastases from colon cancer identified in the abdomen, but outside the liver, at the time of operation. In only 12 (48%) of these patients was the disease identified by the Indacea scintigraphy with the Indacea scans being correctly interpreted. The high number (7) of false positive extrahepatic metastatic findings on Indacea scans resulted in a poor predictive value for a positive scan in this area (63%).

Unlike extrahepatic abdominal metastases, extraabdominal metastatic colorectal cancer was much more reliably identified by Indacea scintigraphy. Ten patients had metastasis from colon cancer documented extraabdominally. Eight (80%) were visualized as positive images by Indacea scintigraphy (Fig. 2).

Tissue Analysis. The greatest tissue content of $^{111}$In was found at the center of primary tumors, in mesenteric lymph nodes that drain primary tumors, and in normal liver (Table 2). Metastases, especially extrahepatic metastases, had less $^{111}$In uptake. Normal tissues, except liver, had the lowest Indacea uptake.

The mean CEA content of primary tumors, hepatic metastases, and tumor-bearing mesenteric lymph nodes was very high (>25 µg/g). The mean CEA content of extrahepatic metastases, benign adenomas, and normal liver was intermediate (15 to 20 µg/g). The mean CEA content of normal intestine and histo-
Fig. 2. Hepatic metastases, mediastinal metastasis. A, scintiscan of anterior chest and upper abdomen, in a 26-year-old female 72 h following administration of Indacea (ZCE025, 40 mg; \textsuperscript{111}In, 4.7 mCi). The patient had had a sigmoid resection 18 months previously for primary carcinoma of the colon and systemic chemotherapy since that time for multiple hepatic metastases. Progression of hepatic disease was documented by CT scan. Indacea scintiscan showed multiple filling defects (photopenic areas, white arrows) in the liver (L). Two areas of increased uptake were seen in the upper mediastinum (black arrows) suggestive of metastases. B, CT scan of the chest demonstrating a 1.5-cm mass (T) in the region of the aorticopulmonary window and in the upper mediastinum. As a result of the Indacea and CT scan findings no surgical procedure was undertaken for the hepatic metastases. The patient received further systemic chemotherapy and has gone on to demonstrate progressive pulmonary metastases.

Fig. 3. Retroperitoneal lymph node. Scintiscan of posterior abdomen and pelvis in a 51-year-old female 7 days following administration of Indacea (ZCE025, 40 mg; \textsuperscript{111}In, 5.7 mCi). The patient had had a previous right hemicolectomy for colon cancer. Over several months the plasma CEA rose from <2.5 to 11.6 ng/ml. The Indacea scan showed increased uptake in the upper abdomen interpreted as either metastatic colon cancer in the caudate lobe of the liver (L) or celiac lymph node (LN) metastasis. At laparotomy, a metastasis in the dome of the right lobe of the liver was resected. No tumor was identified in the caudate lobe of the liver or in the retroperitoneal and perihepatic tissues. Insufficient tissue was available for \textsuperscript{111}In and CEA tissue analyses. The area of increased uptake in the upper abdomen probably represented concentration of Indacea in a histologically normal lymph node draining the right lobe of the liver. The patient remains without evidence of recurrent disease 12 months following partial hepatectomy and placement of a pump/catheter for hepatic infusional chemotherapy.

Table 2 Tissue analysis of tumor and normal tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Biodistribution (mean % ID/kg ± SE)*</th>
<th>CEA content (mean ng/g ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumor (rim)</td>
<td>10.8 ± 1.5</td>
<td>29.0 ± 3.5</td>
</tr>
<tr>
<td>Primary tumor (center)</td>
<td>16.9 ± 10.3</td>
<td>67.3 ± 14.7</td>
</tr>
<tr>
<td>Tumor containing mesenteric lymph node</td>
<td>20.3 ± 4.0</td>
<td>112.2 ± 31.2</td>
</tr>
<tr>
<td>Hepatic metastasis (rim)</td>
<td>11.3 ± 2.1</td>
<td>37.0 ± 7.4</td>
</tr>
<tr>
<td>Hepatic metastasis (center)</td>
<td>6.2 ± 2.9</td>
<td>88.7 ± 29.9</td>
</tr>
<tr>
<td>Extrahepatic metastasis</td>
<td>3.0 ± 0.7</td>
<td>17.2 ± 5.9</td>
</tr>
<tr>
<td>Benign adenoma</td>
<td>2.4 ± 0.2</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>Bowel wall</td>
<td>1.3 ± 0.2</td>
<td>9.9 ± 2.2</td>
</tr>
<tr>
<td>Normal mucosa</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Fat</td>
<td>18.5 ± 1.8</td>
<td>12.1 ± 1.7</td>
</tr>
<tr>
<td>Normal mesenteric lymph node</td>
<td>20.3 ± 1.7</td>
<td>17.0 ± 4.2</td>
</tr>
</tbody>
</table>

* % ID/kg, percentage of injected dose of \textsuperscript{111}In/kg of tissue.

logically normal mesenteric lymph nodes draining a primary tumor was low (5 to 15 μg/g). The lowest mean CEA content was found in fat. The effectiveness of imaging primary tumors was correlated with tissue analysis parameters (Table 3). The tumors that were visualized had significantly more \textsuperscript{111}In (P < 0.005), had significantly higher CEA content (P = 0.05), and were significantly larger (P < 0.05) (all using the Wilcoxon rank-sum test).

Clinical Relevance. Of the 108 evaluable preoperative Indacea scans, 56 were from patients that presented with known or suspected hepatic metastasis and no evidence of unresectable extrahepatic metastatic disease by conventional investigation (X-rays, CT, magnetic resonance imaging, nuclear medicine) or from patients suspected of having recurrent colorectal cancer because of an elevated plasma CEA, but without localization of recurrence site by conventional investigation. Twenty (36%) of these 56 patients were documented to have extrahepatic metast-
tases by the subsequent surgical procedure. In 10 (50%) of these 20 patients the extrahepatic disease was visualized on the Indacea scan and correctly interpreted. In 8 patients the disease correctly identified was extraabdominal. One patient had disease correctly identified at both sites. In 6 of the 56 patients (11%), the Indacea scan proved to have a false positive interpretation. In 8 patients the disease was originally hypothesized that antibody imaging could be used to identify nodal metastases preoperatively. Unfortunately, although histologically positive nodes contain substantial CEA and have good uptake of Indacea, these nodes are usually too small to be visualized. On the other hand, histologically negative lymph nodes contain much less CEA but have as much or more CEA:Indacea complexes in the blood is responsible for much of this normal liver visualization. The CEA content of extrahepatic metastases tends to be low and as a result Indacea uptake and imaging of extrahepatic metastases are also low. Examination of primary tumors indicates that tumor imaging is associated with high uptake of Indacea, large tumor size, and high CEA content (Table 3).

Perhaps the most interesting tissue analysis findings relate to the mesenteric lymph nodes that drain primary tumors. It was originally hypothesized that antibody imaging could be used to identify nodal metastases preoperatively. Unfortunately, although histologically positive nodes contain substantial CEA and have good uptake of Indacea, these nodes are usually too small to be visualized. On the other hand, histologically negative lymph nodes contain much less CEA but have as much or more CEA. We have seen that these nodes can lead to false positive scan interpretation (Fig. 3) and we hypothesize that they represent concentration of Indacea by the lymphatics that drain a tumor where the Indacea has accumulated.

The identification of hepatic metastases by Indacea scintigraphy is comparable to conventional liver scintigraphy using labeled colloid or albumin. The imaging is nonspecific and lesions are usually seen as filling defects. A clinical role for Indacea scanning of hepatic metastases must await methods to decrease the nonspecific uptake of Indacea by normal liver.

The low sensitivity (48%) of Indacea scintigraphy for extrahepatic abdominal metastases, the relatively high false positive rate (7 of 83, 8%), and the low predictive value of a positive scan (63%) are disappointing and indicate the need for caution in interpretation of Indacea scans for extrahepatic abdominal disease.

The identification of extraabdominal metastases by Indacea scanning was more reliable than identification of abdominal (hepatic or extrahepatic) metastases (Table 1). Although the numbers were small, the Indacea scan had its most important impact in this area, often leading to further investigations and major changes in management decisions (Fig. 2).

Analysis of resected tissues has provided some reasons for these imaging successes and shortcomings. Primary tumors tend to be small (relative to hepatic metastases) but have a high content of CEA and good uptake of $^{111}$In (Table 2) accounting for their effective imaging with Indacea. Hepatic metastases also have high content of CEA and reasonable uptake of Indacea but are not well visualized because of the high normal liver uptake of Indacea. Normal liver does contain measurable CEA that may account for some of this uptake, but preliminary results in the nude mouse model suggest that the formation of CEA:Indacea complexes in the blood is responsible for much of this normal liver visualization. The CEA content of extrahepatic metastases tends to be low and as a result Indacea uptake and imaging of extrahepatic metastases are also low. Examination of primary tumors indicates that tumor imaging is associated with high uptake of Indacea, large tumor size, and high CEA content (Table 3).

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The identification of peritoneal, retroperitoneal, and pelvic metastases by CT scan in high risk patients has been disappointing (15, 16). In 17 of 56 patients (30%) with known or suspected hepatic metastases and no evidence of other abdominal/pelvic disease by conventional studies, recurrent extrahepatic intraabdominal cancer was documented at celiotomy. Operative inter-
extraabdominal metastasis was identified by Indacea imaging.

This highly selected but high risk population of patients, Indacea scintigraphy identified 8 of 17 patients (47%) with extrahepatic disease, identified a significant (16 of 56, 29%) role for Indacea immunoscintigraphy in the preoperative workup of colorectal cancer patients with known or suspected hepatic metastases, and no known extrahepatic disease, identified a significant (16 of 56, 29%) subgroup that were at very high risk (10 of 16, 63%) for the presence of extrahepatic disease. The management of each of these ten patients was, or could have been, modified by the scan findings. In some patients and in several others ineligible for this series (Fig. 2) unnecessary surgery was eliminated as a result of Indacea scan findings. In short, a clinically relevant role for Indacea immunoscintigraphy in the preoperative workup of colorectal cancer patients with known or suspected hepatic metastases has been documented. This role complements conventional imaging techniques such as CT and magnetic resonance imaging for identification of extrahepatic and extraabdominal metastases.

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References

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